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Probiotics and prebiotics - renaissance of a therapeutic principle

Premysl Fric*

Department of Medicine, First Faculty of Medicine, Charles University and Central Army Hospital, Prague, Czech Republic

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Abstract: Probiotics are nonpathogenic microorganisms mostly of human origin which, when administered in adequate amounts, confer a health benefit on the host and enable to prevent or improve some diseases. Probiotics may be a natural temporary constituent of the resident intestinal microflora, but their concentration is not sufficient for therapeutic purposes. The microbiota, the intestinal epithelium, and the mucosal immune system constitute the gastrointestinal ecosystem. All three components are essential for complete functional and developmental maturity of the system. The viability of intestinal microflora (including probiotic strains) requires the availability of nutritional substrates (prebiotics), i.e. various types of fiber and oligosaccharides. Prebiotics are cleaved by microbial enzymes to numerous substances (short-chain fatty acids, aminoacids, polyamines, growth factors, vitamins and antioxidants) indispensable for metabolic and functional activities of the intestinal mucosa. The principal probiotics in use include lactobacilli, bifidobacteria, some nonpathogenic strains of *Escherichia coli*, and *Saccharomyces boulardii*. These microbiota display favourable effects which qualify them for therapeutic use. For this purpose, probiotics have to fulfill a series of requirements verifying their efficacy and safety. Experimental and clinical studies examine the prerequisites for the administration of probiotics in digestive diseases, allergic and atopic affections, as well as in some extraintestinal conditions. Future goals of probiotic application include genomic analysis, controlled postnatal colonisation of the digestive tract, the use of probiotics as carriers of peroral vaccines, and recombinant probiotics with *in-situ* production and targeted application of therapeutic molecules.

Keywords: Gastrointestinal ecosystem, probiotics, prebiotics, synbiotics, microbial interference therapy

* E-mail: fricprem@uvn.cz

1 Introduction

1.1 Gastrointestinal Ecosystem - Function and Significance

The intestinal microbial flora, epithelium, and the mucosal immune system constitute a highly integrated unit called the gastrointestinal ecosystem (GES). Individual components may develop to a certain degree separately, but full morphological and functional maturity of the digestive system requires the presence of all three components. The components have their inherent functions, numerous mutual interactions and a complicated equilibrium exists among them. The outcome is modeling of development, morphology, and functions of individual components as well as of the digestive system as a whole. Disturbances of the equilibrium due to inborn (genetic) or acquired alterations of any component may result in pathological changes of the digestive system.

1.2 Resident Microflora (MF)

The term indicates microbial flora of the healthy intestine. In the past intestinal MF has been followed almost exclusively in pathologic relations to the host, i.e. in association with infectious diseases. Until recently, knowledge concerning the composition and function of resident MF has been very limited. The system comprises approximately 400 various species of microbes with a total of about 10^{14} prokaryotic organisms, which is 10-times more than the total number of eukaryotic cells of the human body [1]. Only $20-40$ percent of microbial strains can be detected by the special cultivation techniques. Only recently introduced methods of molecular microbiology (16S RNA and rDNA determination) facilitate identification of other species within this complex population of microorganisms [2]. Similarly, molecular immunology and biochemistry enlarged our knowledge of the intestinal MF. At present we consider intestinal MF a *postnatally acquired organ* with important physiologic, metabolic, and immune functions. The scope of metabolic processes produced or controlled by intestinal MF is comparable with liver metabolism.

The fetal digestive tract is sterile. Its microbial colonization from surrounding environment starts with passage of the foetus through the birth channel. Breast-fed, fully matured newborns acquire more suitable MF with predominant bifidobacteria. In bottle-fed newborns coliform microbes, enterococci and bacteroides are the prevailing strains. Newborns at intensive care units are colonized at a slower rate and bifidobacteria appear in the intestines later [3]. These alterations may influence the acquisition of some diseases (e.g. enterocolitis).

The development of MF is a phased process dependent on the composition of the food. The first microbes that colonize the intestine, are aerobic and facultative aerobic strains such as *Escherichia coli*, lactobacilli and streptococci. The decrease of the oxidation-reduction potential by these strains enables colonization by anaerobic bacteria such as bacteroides and bifidobacterium. After introducing solid food, composition of MF gradually approaches the definite state with prevalence of anaerobes. Intestinal MF

in an adult person is relatively stable, but significant differences among individuals may be found. In the same subject changes of MF may occur as a consequence of disease, antibiotic administration and alterations in food composition.

MF differs in individual segments of the digestive tract both quantitatively and qualitatively (*proximodistal gradient*). The proximal small intestine contains mainly microbes from the upper parts of the digestive tract. Composition of MF of the distal small intestine is similar to that of the colon and, moreover, segmental filamentous bacteria are present. In the large bowel, anaerobes such as Bacteroides, Eubacterium, Streptococcus, Fusobacterium and others, are prevalent. The ratio of anaerobes increases up to 99% in the rectum. However, MF of the colon is also stratified horizontally. There is a difference between the luminal and mucosal MF, which is further divided into flora of the mucus layer, crypts and flora adhering to colonocytes [4–6].

1.3 Intestinal Epithelium

Multipotent stem cells present at the bottom of Lieberkühn's crypts reproduce quickly and differentiate into enterocytes, goblet cells, Paneth cells, endocrine cells, and so-called M cells. Paneth cells concentrate on the basis of the crypts while cells of other types migrate from the crypts along the wall of villi of the small intestine or epithelial surface cuff of the large bowel. The life cycle of intestinal epithelial cells takes $2-5$ days and apoptotic cells are exfoliated into the lumen. Paneth cells survive up to 20 days and are removed by phagocytosis [7, 8].

Enterocytes represent a massive physical barrier that becomes fully functional by closing-up the cell membranes within 48 hours after birth and thus exposure of the mucous membrane to antigens is decreased. Mucins, lysozyme and defensins, produced by the intestinal epithelia, inhibit the growth of microbes and their adherence to cells. At the same time they produce a number of interleukins (IL-1α, IL-1β, IL-6, IL-8, IL-10), TNF-α, TGF-β, and some chemokines (MIP-3α, TARC). Thus, enterocytes should be considered as key elements in integrating intestinal MF with local intestinal defense and development of the host as well as important participants in interactions among epithelium, mesenchyme, and immune system [9, 10].

1.4 Mucosal Immune System (MIS)

The intestinal compartment of MIS includes an inductive (Peyer's patches) and effector (mucosal lamina propria and intraepithelial spaces) site. Antigenic substances that are not inactivated by certain intestinal barrier mechanisms pass the epithelium by several mechanisms: via M cells, across the brush border of enterocytes, and paracellularly across tight junctions [11–13]. All these mechanisms include a number of delicate, complex, and coordinated processes.

The main proportion of luminal antigens is transported via M cells, i.e. modified enterocytes capable of pinocytosis. These are localized at sites of underlying lymphatic

follicles (Peyer's patches), in which antigen presentation and lymphatic cell activation occurs. Activated antigen-specific lymphocytes migrate to the mesenteric lymph nodes and possibly via the ductus thoracicus into blood circulation. These migrating lymphocytes express specific mucosal receptors (homing receptors) by means of which they enter into effector sites of the intestinal MIS (lamina propria, intraepithelial spaces). The lamina propria includes plasmocytes (antibody-producing B lymphocytes), T lymphocytes, and macrophages. Plasmocytes produce large amounts of secretory IgA that is able to bind antigens in the lamina propria and intestinal lumen. It does not activate the complement system and in this way prevents an excessive inflammatory response to the presence of antigens. T lymphocytes in lamina propria are mostly CD4+ helper T cells expressing TCR$\alpha\beta$. Intraepithelial lymphocytes are mostly CD8+ helper T cells expressing TCR$\gamma\delta$ with cytolytic activity [14].

Antigens of lower molecular mass are transported mainly across the brush border of enterocytes. These peptides are split by lysosomal hydrolases into small nonimmunogenic peptides. The remaining fraction (about 1%) is absorbed paracellularly across tight junctions as intact molecules. This process is subject to a sophisticated control and under physiological conditions results in a specific immune response leading to tolerance of the antigen (tolerogenic response). An important role in regulation of this process is played by zonulin, an endogenous structural analogue of the zonula-occludens toxin of *Vibrio cholerae*, located at tight junctions [15–18]. Increased activity of this peptide is presumed to be at least partly responsible for the increased permeability of tight junctions with subsequent production of antibodies to intestinal antigens (immunogenic response).

2 Probiotics and microbial interference therapy

Probiotics are nonpathogenic microorganisms mostly of human origin which, when administered in adequate amounts, confer a health benefit on the host and are able to prevent or improve some diseases. Probiotics may be considered as *imported commensal microflora*. The first to discover and to use the probiotic principle was I. I. Metchnikoff (1845-1916), the Russian microbiologist and Nobel-prize laureate. Metchnikoff is the author of the concept of antibiosis, i.e. suppression of growth and other life phenomena of one microorganism by another. This concept was confirmed by Fleming's discovery of penicillin and the following antibiotic era. Metchnikoff himself envisaged its implementation in the *microbial interference therapy*. At the beginning of the past century, Metchnikoff isolated *Lactobacillus bulgaricus* from fermented milk. Metchnikoff considered this milk to be the reason for longevity of Bulgarian farmers. Despite formal imperfections, the rationale of microbial interference therapy remains intact. Its core idea is administration of living microorganisms to alter and stabilize the resident intestinal MF with a subsequent favorable effect on the health status of the host and in this way to prevent some diseases or improve their course.

Probiotics include mainly lactobacilli and bifidobacteria. These microbiota may be present in intestines of healthy individuals and their consumption in fermented milk products favorably affects human health. Probiotic effects were also demonstrated in clinical trials using some strains of the genera Escherichia, Enterococcus, Bacillus, and Saccharomyces. Any microorganism classified as "probiotic" has to prove efficacy and safety under recommended conditions for use in a defined population, methods of application and dosage [19]. Even food supplements of microbial origin have to fulfill the criteria of the European Union [20].

2.1 Requirements for Probiotics and Their Properties

Microorganisms used as probiotics have to fulfill the following requirements:

(1) Detailed definition and typing,
(2) Absence of any pathogenic characteristics (including production of enterotoxins and cytotoxins, enteroinvasivity, pathogenic adhesion, hemolysis, serum resistance, serum pathogenicity, presence of genes of antibiotic resistance),
(3) Resistance to gastric acid and to bile,
(4) Ability to adhere to the intestinal epithelium,
(5) Ability to colonize the colon,
(6) Clinically proven beneficial health-effects,
(7) Safety [21].

Human origin of a probiotic and its administration in living form are only relative criteria. *Saccharomyces boulardii*, the probiotic character of which has been sufficiently proven, has no human origin. It is probably more suitable to name it a biotherapeutic agent. Antiinflammatory effects of probiotics in experimental colitis may be mediated by microbial components such as peptidoglucan, lipopolysaccharide, and nonmethylated DNA [22, 23].

Increasing attention is paid to detailed typing of probiotics. DNA-DNA hybridization or sequencing DNA regions encoding species-specific areas of 16S rRNA are used to test species classification. These techniques are combined with specific cultivation methods for verifying the microbial phenotype.

Genomic analysis is of principal importance for the detailed knowledge of individual probiotics. The first probiotic with knowledge of the whole genome was *Lactococcus lactis* subsp. IL1403 [24]. This was followed by the complete genomic analysis of *Lactobacillus plantarum* [25], *Bifidobacterium longum* [26], and *Escherichia coli* Nissle 1917 [27, 28]. Full genomic analysis can be shortly expected in further lactobacilli (*L. johnsoni, L. acidophilus, L. gasseri,* 2 strains of *L. casei,* and *L. rhamnosus*), bifidobacteria (second strain of *B. longum* and *B. breve*), and *Saccharomyces boulardii*. Nevertheless, genomically non-defined microorganisms (e.g. *L. casei Shirota, L. reuteri, Streptococcus salivarius* subsp. thermophilus, and nonpathogenic *E. coli* O83:K24:H1) or even mixtures of microbes are also employed as probiotics. Genomic analysis is indispensable for predicting the effects of individual probiotics as well as for studying the relationship between probiotics and

prebiotics and life conditions of the intestinal MF. The final goal of these studies is to create a global genome bank of intestinal procaryotes [29]. Molecular genetics create also prerequisites for recombinant studies aimed at suitable changes of the genome and functions of probiotics *in vivo* [30, 31].

The effects of probiotics are based on a variety of properties:

(1) Competition with pathogenic microbes for adherence to intestinal epithelium [32–34],
(2) Synthesis of peptides with bacteriostatic and bactericidal activity (colicins, microcins [35],
(3) Regulation of intestinal barrier function and microbial translocation [36–39],
(4) Modulation of function of intestinal epithelia and dendritic cells [40–43],
(5) Influence on local and systemic immune response [44–48],
(6) Inhibition of pathogen overgrowth [49],
(7) Stimulation of toxin elimination [50],
(8) Synthesis of steroids from cholesterol [51, 52],
(9) Influence on secretion of mucus, absorption, motility, and splanchnic blood flow [53].

2.2 Probiotics - Induction of Immunity and Immune Response

Both the resident and imported MF display immunostimulatory activity. However, the extent and duration of immunostimulatory activity and immunomodulation are different. Resident MF constitutes a permanent association with the host (*autochtonous MF*). Probiotics are present in the intestines during and shortly after administration (up to several weeks). Their association with the host is only temporary (*allochtonous MF*). The reason for the difference is unknown. Presumably, the host cells do not express permanent receptors for probiotics or the probiotic is unable to compete permanently with the autochtonous MF for close contact to the epithelium. Immune cells display recognition receptors for probiotics. It is not known, however, whether persistence and replication of imported MF are necessary for the induction of the immune response, or whether contact with antigen alone is sufficient for triggering the signaling cascade that leads to activation of immune cells in lamina propria with subsequent upregulation of cytokines and bioactive molecules.

Colonization of the intestine with resident MF is of critical importance for the development of oral tolerance. This process is realized after birth more or less at random and the response to environmental antigens in newborns is shifted in favor of the Th2 cytokine profile, which is typical for allergic diseases. Insufficient exposure of the intestine to MF, under normal circumstances necessary for a balanced maturation of the immune system into nonatopic state, is considered to be a probable cause of the increased frequency of allergic diseases in developed countries [54, 55]. Targeted postnatal colonization with probiotic strains may be considered to improve the present situation [56–59]. It remains an open question whether with this procedure probiotics might become constituents of the autochtonous MF.

2.3 Probiotics - Barrier Function and Microbial Translocation

The intestinal mucosal barrier regulates transport processes between the intestinal lumen and internal environment. Individual absorption mechanisms are gently regulated by means of membrane pumps, ion channels, and tight junctions. Tolerance to commensal MF and immune reactions to pathogens require intact uptake, recognition, and processing of antigens as well as an adequate immune response. Disturbance of these processes, particularly disturbance of microbial translocation due to increased permeability and failure of oral tolerance caused by inadequate interaction between the intestinal epithelium and T lymphocytes, may result in inflammation and tissue lesion.

Processes associated with adhesion of resident MF and probiotics to the intestinal epithelium have been elucidated so far only partially. Adhesion of probiotics to the intestinal epithelium varies according to the type of probiotic rather than according to the animal species [60]. Under experimental conditions, probiotics favorably influence the intestinal barrier and lower the activity of mucosal inflammation, including decreased secretion of TNFα and IFNγ [36]. Similarly, *Lactobacillus plantarum* regulates enhanced permeability of the intestinal barrier caused by pathogenic *E. coli* [38], and *Bifidobacterium lactis* significantly lowers the risk of bacterial translocation in experimental short bowel syndrome [37].

Translocation of commensal MF into mesenteric lymph nodes has been proven. This process is decisive for the activation of the intestinal immune system [61] and may influence the overall stimulatory effect of probiotics. On the contrary, epithelial adhesion may induce more specific effects [19]. The effects of probiotics in relation to translocation and adhesion to various types of intestinal epithelia will be possibly elucidated in more detail with the use of recombinant probiotics.

3 Prebiotics

Prebiotics are substances not cleavable by enzymes of human gut cells but possibly becoming substrates of enzymes of the prokaryotic microorganisms inhabiting the digestive tract or other organs (e.g. oral cavity, skin, and vagina). Prebiotics include various oligosaccharides (fructo-, gluco-, galacto-, isomalto-, xylo-, and soyooligosaccharides), lactulose and lactosucrose. Another significant and widely used prebiotic is fiber and its split products (pectins, xylans, and cellulose). In humans, prebiotics should constitute about 10% of total energetic intake and about 20% of the volume of total food intake. Presumably, such balanced food designated also as “functional food”, is able to control and regulate various body functions, contribute to good health status, and decrease the risk of some diseases. Enzymatic conversion of prebiotics yields a wide range of substances such as short-chain fatty acids (particularly butyric acid), some amino acids (e.g. arginine, cystein, and glutathione), polyamines, growth factors, vitamins, and antioxidants. These substances cover a significant part of nutritional needs of the colonic mucosa and participate in a number of metabolic processes. Butyrate is the main energetic source of

colonocytes. Oligofructose and inulin stimulate growth and activity of bifidobacteria and lactobacilli without inducing similar changes in anaerobes, clostridia, coliform flora, and the genus Bacteroides [62, 63]. Oligofructose partially prevents the decrease of intestinal short-chain fatty acids in subjects on enteral nutrition [64].

4 Synbiotics (Eubiotics)

These terms designate mixtures of probiotics and prebiotics that mutually modulate intestinal MF in favor of the host starting right at birth. These mixtures may have beneficial effects on the balanced state of the MF. Under experimental conditions, oligofructose-enriched inulin stimulates IL-10 secretion in lymphocytes of Payer's patches and secretion of secretory IgA in the ileum. It may be assumed that prebiotics primarily influence the intestinal immune system. Probiotics alone (*Lactobacillus GG* and *Bifidobacterium*) displayed in the same experiment only mild alterations of immune functions. Simultaneous administration of probiotics and prebiotics increased secretory IgA in the ileum and lowered oxidative activity of neutrophils. It appears that synbiotics may have effects different from those found when probiotics and prebiotics are used separately, and the outcome may not represent a simple additive or synergistic effect [45].

5 Probiotic therapy

In the last fifteen years, therapeutic use of probiotics has been the subject of a great number of both experimental and clinical studies of various design. They yielded new knowledge on the topic. This review includes studies that represent an advance or indicate probable direction of further development.

6 Experimental studies

6.1 Experimental Colitis and Enterocolitis

The pathogenic role of MF in the development of colitis was demonstrated by its occurrence in conventional immunodeficient (SCID) mice after one-week administration of dextransulphate, whereas in germfree SCID animals no histological changes of intestinal mucosa were observed [65]. Various probiotics have been used in different models of experimental colitis. In interleukin-10 deficient homozygous mice (IL-$10^{-/-}$) colitis can be prevented by administration of *Lactobacillus reuteri* [66]. Recombinant IL-10 producing *Lactococcus lactis* has similar effects. In addition, this probiotic mitigates colitis in conventional mice [30]. *E. coli* strain Nissle 1917 has beneficial effects on chronic colitis induced by transfer of CD4+ and CD62L+ T-lymphocytes to immunodeficient SCDI mice. It also decreases proinflammatory cytokines without histological improvement in conventional mice with dextransulphate induced colitis [67]. In addition, this probiotic produces an antiinvasive substance inhibiting various intestinal pathogens (e.g. *Salmonella*

typhimurium, Shigella flexneri, Yersinia enterocolitica, Legionella pneumophila, and *Listeria monocytogenes*) without direct contact of the probiotic with these pathogens or the intestinal epithelium [68]. This observation may be associated with expression of the antimicrobial peptide defensin-2 in human intestinal epithelium, which is induced by *E. coli* Nissle 1917 as well as by some strains of lactobacilli [69]. Colitis induced by *Bacteroides vulgatus* in transgenic HLA-B27 rats can be cured with antibiotics (vancomycin and imipenem). The disease will relapse after antibiotics are stopped. *Lactobacillus GG* will not prevent the onset of colitis and will not cure established inflammation. It will, however, prevent relapse of colitis in antibiotic-treated animals [70]. These findings suggest that probiotics are probably more effective in preventing relapse than in inducing remission. Sequential administration of antibiotics and probiotics may display an effective long-term therapeutic approach in intestinal inflammatory conditions [71]. The effects of probiotics also differ according to the model of experimental colitis. Mixed probiotic VSL#3 and *Lactobacillus GG* significantly improve colitis induced by iodoacetamide, which inactivates SH groups. These probiotics are, however, inactive in immune-mediated colitis induced by dinitrobenzenesulphate. The findings point to the possible role of SH compounds in the protective effect of some probiotics [72]. *Lactobacillus GG* significantly suppresses internalization of enterohemorrhagic *E. coli* in tissue culture [34, 73]. This effect is mediated by high adhesivity of the probiotic to colonocytes and by MUC-3 gene activation with subsequent mucus secretion [74]. *Saccharomyces boulardii* contains a protease inactivating the epithelial receptor of *Clostridium difficile* toxin A and B. The yeast also produces polyamines used by the colonocytes for proteosynthesis and maturation [75, 76]. *Lactobacillus acidophilus* and *Lactobacillus reuteri* mitigate the development of Cryptosporidium enteritis in immunodeficient mice [77]. *Lactobacillus casei* DN-114001 and *Bacteroides thetaiotaomicron* inhibit rotavirus infection in HT-29 colonocytes [78].

6.2 Experimental Colorectal Cancer

The effects of live probiotics, their cellular components and metabolites have been followed in animal models and tissue cultures. Azoxymethane, dimethylhydrazine and heterocyclic aromatic amines have been used to induce tumour changes. Various strains of lactobacilli, bifidobacteria and *Streptococcus thermophilus* have been administered most frequently. The changes comprised a decreased number of aberrant crypts, diminished proliferative activity of the mucosa, and the decline of p21 oncogen in the tumor and surrounding mucosa [79–81]. Probiotics may be able to inhibit cancerogenesis by various mechanisms: binding of cancerogens, production of detoxifying substances, activation of detoxifying enzymes (NADPH-cytochrom 450 reductase and glutathione S-transferase), induction of changes of intestinal MF, increased production of nutritional substances (e.g. short-chain fatty acids), regulation of motility, control of life-cycle of colonocytes, and stimulation of the mucosal immune system [82–84]. Prebiotics play a significant role in this experimental model as well. Isolated administration of prebotics (xylo- and fructooligosaccharides) enhances the population of bifidobacteria and lowers the pH of intestinal contents as

well as the number of aberrant crypts [85]. Simultaneous administration of probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium lactis*) and prebiotics (inulin enriched with oligofructose) extends the inhibitory effect by modulating the functions of the intestinal immune system (e.g. stimulation of IL-10 production and decrease of IFNγ in Payer's patches) [86].

6.3 Liver Diseases

In cirrhotic rats the small intestine is contaminated with colonic microflora and the intestinal barrier is disturbed. The microbiota pass at increased rates into mesenteric lymph nodes and are the main cause of ascites infection and spontaneous bacterial peritonitis. Identity of microbial strains in the intestine, mesenteric nodes, and ascites was proven by DNA analysis [87, 88].

Short-term administration of *Lactobacillus GG* for $8 - 10$ days does not prevent translocation of the intestinal flora, although the probiotic colonized the cecum in 90% of cirrhotic rats [89]. On the contrary, preventive administration of *Lactobacillus plantarum* to healthy rats for one week inhibits the enhancement of permeability following administration of *E. coli* [38].

Nonalcoholic steatosis of obese mice includes intestinal bacterial overgrowth and increased expression of TNFα. VSL#3 or anti-TNF antibodies improve liver histology and decrease concentration of fatty acids in the liver as well as activity of alaninaminotransferases in the serum. These changes are accompanied by a decreased expression of liver mRNA TNF in mice treated with anti-TNF antibodies but not with the probiotic alone. Combination of both preparations decreased expression of Jun N-terminal kinase, which is regulated by TNF and induces insulin resistance. Treatment improved insulin resistance and decreased β-oxidation of fatty acids in the liver. It may be suggested that in non-alcoholic steatosis of the liver intestinal MF induces endogenous signals participating in the pathogenesis of insulin resistance [90].

6.4 Acute Pancreatitis

Mangiante *et al.* [91] administered *Lactobacillus plantarum* to rats perorally (5 ml with $0.5 - 1 \times 10^9$ CFU), 4 days prior to and 4 days after inducing acute pancreatitis by ligating the common biliopancreatic duct. Intestinal flora constituents (*E. coli, Enterococcus faecalis,* and the genera Pseudomonas and Proteus) were detected three times more frequently in mesenteric lymph nodes and in pancreatic tissue of control animals not supplemented with probiotics. Akyol *et al.* [92] induced acute pancreatitis in rats by taurocholate injection and started therapy after 6 hours. Ciprofloxacin, meropenem and *Saccharomyces boulardii* were administered either separately or as a combination of the probiotic with one or both antibiotics. Histopathological changes were of lower intensity in all treated groups as compared to controls, but a significant difference was found only in the group with combined administration of the probiotic and ciprofloxacin. It may be

suggested that in acute pancreatitis the prerequisites for therapeutic effects of probiotics include preventive or very early application.

6.5 *H. pylori* Infection

Lactobacillus rhamnosus and *Lactobacillus acidophilus* administered to mice prior to and after experimental infection significantly decreased the number of infected animals (100% vs. 50%). Antral gastritis was less pronounced in animals with preventive administration of probiotics [93].

6.6 Syndrome of Multiorgan Dysfunction

Conventional and IL-10-deficient mice were pretreated with VSL#3. Subsequently, sepsis with multiorgan dysfunction was induced by intraperitoneal injection of lipopolysaccharide and D glucosamine. Barrier function of the intestinal mucosa remained intact in the group pretreated with probiotics and secretion of proinflammatory cytokines was lower than in controls. Probiotics may deserve attention as adjunct therapy of risk patients in intensive care units [94].

7 Clinical studies

7.1 Inflammatory Bowel Disease (IBD)

Failure of immunological response to antigens from intestinal contents (loss of immunological tolerance) in genetically predisposed persons is considered the basis of IBD pathogenesis. The mucosal compartment of intestinal MF appears to play the triggering role. In IBD subjects this MF is very numerous, adheres to the mucosa. Intracellular inclusions of polymorphic bacteria appear with high concentrations in epithelia at the basis of the crypts without direct contact with the intestinal flow [6, 95].

Some microbial products such as peptidoglucan, lipopolysaccharide, and microbial DNA (CpG) are selectively bound to the membrane receptors (toll-like receptors, TLR1 - TLR9) or cytoplasmic receptors (NOD1 and NOD2) that activate the nuclear factor κB and transcription of proinflammatory cytokines, adhesive, costimulatory, and MHC II. class molecules. The behavior of the resident MF in IBD subjects has changed. Some strains become able to cause disease in genetically predisposed persons, some behave neutrally, and some may have a protective effect. Aggressive strains include the genera Bacteroides, Eubacterium, Peptostreptococcus, *Enterococcus faecalis*, enteroinvasive *E. coli,* Pseudomonas, and *Fusobacterium varium.* Protective effects may be displayed by lactobacilli, bifidobacteria, and some nonpathogenic strains of *E. coli.* The inflammatory response is the result of genetic predisposition, loss of immunological tolerance, and the behavior of mucosal MF [23].

The present pharmacotherapy of IBD is concerned almost exclusively (with the exception of antibiotics) with the blockade of inflammatory and immunological responses. This approach is aimed more at consequences than at causes of the pathogenic processes. The optimal therapy, however, requires to consider also prevention and recurrences of IBD. Prevention of IBD demands elimination of the dominant antigens and blockade of the immunological responses to these stimuli. Antibiotics lower or eliminate pathogenic microbes, probiotics increase the protective mechanisms [71, 96–98]. Antibiotics have definite indications in the treatment of acute inflammatory processes and complications of IBD (e.g. abscesses, fistulas, contaminated small intestine, postoperative infections, toxic megacolon, and secondary infections). Antibiotics are, however, unsuitable for the prevention of IBD in view of their short-term influence on intestinal MF, considerable undesirable effects, risk of pathogens overgrowth, and development of resistance [99].

The role of probiotics in IBD includes:

(1) Inhibition of aggressive MF (reduction of pH in the intestinal lumen, secretion of bacteriostatic and bactericidal peptides, and competition with aggressive strains for receptors and inhibition of their epithelial invasion);
(2) Improvement of epithelial and mucosal barrier functions (elaboration of short-chain fatty acids, induction of mucus secretion, and reduction of the barrier permeability),
(3) Modulation of MIS responses (induction of expression and secretion of IL-10 and TGFβ, stimulation of secretory IgA production, and decrease of TNFα expression) [100].

The first reports on the use of probiotics in IBD therapy were published in the ninetieth of the past century [101–103]. Since then the topic has remained the object of lasting interest documented by new original works as well as review articles [104].

Idiopathic proctocolitis (IPC) has been the most frequent subject of probiotic therapy. *E. coli* Nissle 1917 has been tested in several double-blind, placebo-controlled studies lasting $3-12$ months in patients with IPC in remission and it was found equally effective as mesalazine in the prevention of relapse [105–109]. Venturi *et al.* [110] applied in a similar indication the mixed probiotic VSL#3 (4 strains of lactobacilli, 3 strains of bifidobacteria, and *Streptococcus salivarius*) to mesalazine-intolerant individuals. 79% of patients remained in remission after one year of treatment. Similarly, Ishikawa *et al.* [111] achieved after one year of treatment with bifidobacteria-enriched milk a significantly lower number of relapses than in the control group. Another probiotic (*S. boulardii*) combined with mesalazine proved effective in mild and moderate relapse of IPC [112]. Tursi *et al.* [113] found that combined therapy with a probiotic (VSL#3) and 5-aminosalicylate allowed for a decrease in the dose of balsalazide by 50% in comparison with monotherapy. It may be suggested that in this combination the probiotic displayed a sparing effect. Furrie *et al.* [114] found in rectal biopsies of IPC subjects already after one-month treatment with *Bifidobacterium longum* and inulin-enriched oligofructose a significant decrease of mRNA for β-defensins, TNFα, and IL-1α. The combination of probiotics and prebiotics may be advantageous for an antiinflammatory effect at least on the molecular level.

In *Crohn's colitis* Malchow [115] observed after induction of remission by prednisolone plus *E. coli* Nissle 1917 that subsequent treatment with this probiotic for one year halved the number of relapses as compared to the placebo group. Guslandi *et al.* [116] compared mesalazine alone and its combination with *S. boulardii* for 6 months in patients with Crohn's colitis in remission. After this time relapses in subjects receiving monotherapy were six times more frequent. In individuals after surgery for Crohn's disease treatment with the nonabsorbable antibiotic rifaximin for 3 months and the probiotic VSL#3 for the next 9 months was followed by only half of relapses in comparison with subjects receiving mesalazine for 12 months (20% vs. 40%) [117].

Probiotics have been successfully used also in the prevention and treatment of *pouchitis*. The topic was studied mainly by Gionchetti *et al.* Acute pouchitis may be prevented by administration of the probiotic VSL#3 immediately after closure of the temporary ileostomy [118]. Another indication is the prevention of relapse of chronic pouchitis after remission achieved by metronidazol and ciprofloxacin [119]. In a double-blind, placebo-controlled study treatment with VSL#3 was followed by relapse in 15% of subjects receiving verum and in 100% of subjects receiving placebo. In addition, all patients relapsed within 3 months of stopping the probiotic [120]. Similar effects were observed after application of lactobacilli [121, 122].

7.2 Infectious Enterocolitis

Probiotics have been frequently used to prevent and treat various intestinal infections such as viral enteritis, some types of bacterial enterocolitis, and nonspecific infections, e.g. traveler's diarrhea. Favorable effects of probiotics are well documented in children with acute diarrheal infections, especially of viral etiology. These conditions were most frequently treated with *Lactobacillus GG, L. plantarum* and *L. casei* Shirota, some strains of bifidobacteria, and *Streptococcus thermophilus* [123–127]. *E. coli* Nissle 1917 and another nonpathogenic *E. coli* strain (O83:K24:H1) prevented diarrhea in sucklings and preterm newborns [56, 128].

Postantibiotic colitis is most frequently caused by toxic strains of *Clostridium difficile*. The condition may threaten mainly polymorbid and immunosuppressed patients, who have a marked tendency to relapse after repeated application of antibiotics (especially cephalosporines, amoxicillin/ampicillin, and clindamycin). The therapy includes metronidazole as the first-choice antibiotic and vancomycin as second choice in case of metronidazole-resistant strains or intolerance. The antibiotics should always be combined with *S. boulardii*. The probiotic is also used for the prevention of relapse in risk subjects [129–133]. *S. boulardii* grows optimally at 37 °C, colonizes the intestine quickly, does not change the resident MF, and after stopping the therapy quickly disappears from the intestine [134]. *Lactobacillus GG* successfully prevented clostridial colitis in children [135, 136].

Traveler's diarrhea is a polyetiological disease with variable incidence in different geographical regions. *Lactobacillus GG* and *S. boulardii* prevented the condition in approximately 50% of individuals at risk [137, 138]. These probiotics are also applied in combination with antibiotics and intestinal disinfectants during the actual illness, but its variable etiology does not exclude success of other probiotics.

7.3 Other Forms of Colitis

E. coli Nissle 1917 was applied in an open study to subjects with *collagenous colitis* for 4 weeks [139]. Significant improvement in the number and consistency of stools was observed. The effect may be related to the inhibitory effect of the probiotic on enteroinvasivity of various intestinal pathogens.

7.4 Irritable Bowel Syndrome (IBS)

The composition of intestinal MF may be altered in some IBS subjects with subsequent changes in nutrient cleavage by microbial enzymes. These alterations include lowering of coliform microbes, lactobacilli, and bifidobacteria in stools, increase of Bacteroides strains, *E. coli*, and anaerobes in intestinal biopsies [140, 141] as well as an increased production of intestinal gas [142]. These findings stimulated the use of probiotics in IBS subjects with the idea of adjusting the intestinal MF. The results differ with regard to the protocol and definition of inclusion and outcome criteria. In a double-blind, placebo-controlled trial *Lactobacillus acidophilus* improved symptoms in approximately half of IBS patients [143], whereas *Lactobacillus casei GG* was found ineffective in a similar study [144] The mixed probiotic VSL#3 decreased abdominal bloating without affecting the number of bowel movements [145]. Combined application of *Lactobacillus plantarum* and *Bifidobacterium breve* for 4 weeks decreased significantly the score of pain as well as other symptoms [146]. *E. coli* Nissle 1917 administered for several weeks displayed significant improvement of chronic functional constipation in two randomized controlled studies [147, 148]. Similar experience was described by Koebnick *et al.* [149] in a double-blind placebo-controlled study with patients suffering from chronic constipation treated for 4 weeks with *Lactobacillus casei* Shirota. Faber [150] used probiotics alone (*Lactobacillus acidophilus* and *Bifidobacterium infantis* for 4 weeks) or in combination with antibiotics (ciprofloxacin during the first week) in three different groups of IBS subjects: with diarrhea, constipation, and alternation of both main symptoms. Both therapeutic approaches improved the quality of life and decreased the frequency of symptoms in all three groups. The complex etiopathogenesis of IBS requires additional studies with detailed description of individual groups of patients and elimination of a possible placebo effect to define more precisely the subgroups of IBS subjects suitable for probiotic therapy.

7.5 Diverticular Disease of the Colon

Symptomatic uncomplicated diverticular disease of the colon occurs in many older subjects with multiple intestinal diverticula. Its symptomatology includes pain in the hypogastrium, irregular bowel movements, abdominal bloating, and excessive flatulence. *E. coli* Nissle 1917 significantly prolongs remission after relapse treated with an intestinal antimicrobial and absorbent [151]. The effect is probably due to the stabilization of intestinal MF, normalization of intestinal dysfunction, and downregulation of hypersensitivity reactions.

7.6 Liver Diseases

Small intestinal bacterial overgrowth occurs in 50 - 70% of subjects with liver cirrhosis. Its main cause is the long-lasting therapeutic suppression of gastric hydrochloric acid. The overgrowth is associated with systemic endotoxemia [152, 153]. This is due to the defect of mucosal barrier with increased translocation confirmed by examination of mesenteric lymph nodes [154–156]. The process correlates with progress of the liver disease and is especially pronounced in patients suffering from severe inflammatory complications [157]. These findings stimulated the idea to use probiotics and prebiotics in addition to antibiotics in *liver encephalopathy* with the presumption of suppressing the MF comprising urease and producing ammonia [158]. Probiotics split nonabsorbable saccharides (e.g. fibre, lactulose) to diarrhea causing products (short-chain fatty acids and carbon dioxide). In this way not only urease-positive microbes but also deaminating strains are removed and intestinal uptake of toxic bacterial metabolites (e.g. ammonia) is reduced. Lactobacilli producing both lactic acid and carbon dioxide from sugars are considered preferable for this purpose [159]. The results of first clinical trials are encouraging [160, 161].

In *nonalcoholic steatohepatitis* probiotics may be part of the therapeutic regimen for the downregulation of inflammatory mediators [162]. Finally, probiotics should be considered a therapeutic modality in *spontaneous bacterial peritonitis* as prophylactic agents and for maintaining remission, or in combination with antibiotics during the acute stage [163, 164].

7.7 Acute Pancreatitis

Olah *et al.* [165] used *Lactobacillus plantarum* (2×10^9 CFU/day) and fibre (Nutrison Fibre®) by nasojejunal tube in subjects with severe acute pancreatitis. The number of patients with infected pancreatic necrosis and abscesses was significantly lower in the group receiving verum.

7.8 *H. pylori* Infection

Some strains of lactobacilli (*L. acidophilus, L. brevis,* LGG) and *Bifidobacterium lactis* reduce signs of *H. pylori* infection in gastric mucosa (decrease of urease and ornithin-decarboxylase activity, increase of polyamines concentration) [166, 167]. An eradication effect of probiotics on *H. pylori* infection has not been proven, but they significantly decrease undesirable side effects of the eradication drugs [168]. This topic requires additional studies aimed at detailed description of probands (asymptomatic volunteers, symptomatic patients), mode of probiotic administration, and outcome definition [169].

7.9 Allergy and Atopy

In newborns, immunologic responses to environmental antigens are deviated toward a Th2-type cytokine profile, which is typical for allergic diseases such as atopic eczema, allergic rhinitis, conjunctivitis, and asthma. Their high prevalence in developed countries is ascribed to excessive emphasis on hygienic measures. This behavior reduces the exposure of newborns to microbial stimuli and immune responses favoring the Th2-cytokine profile persist ("hygiene hypothesis"). Microbial colonization of the intestines is of basic importance for the development of oral tolerance. For this reason an improvement of the present situation might include the application of probiotic strains in early life (controlled or targeted colonization). This concept is supported by a series of clinical studies showing a long-term positive effect. Intestinal colonization of infants with nonpathogenic *E. coli* O83:K24:H1 significantly prevented nosocomial infections [170]. Both this strain and *E. coli* Nissle 1917 increased specific IgA and IgM antibodies [128, 171] and induced nonspecific immune responses in full-term as well as premature infants [44]. A long-term follow-up of children colonized with *E. coli* O83 proved a significantly lower occurrence of allergic conditions after 10 and 20 years as well as of recurrent infections after 10 years [172]. Isolauri *et al.* [57] demonstrated in a double-blind, placebo-controlled study that administration of whey enriched with probiotics (*Bifidobacterium lactis* or *Lactobacillus GG*) to children with atopic eczema for 2 months was followed by a significant improvement of the disease. Kaliomäki *et al.* [173] observed that *Lactobacillus GG* administered to sucklings and breast-feeding mothers halved the frequency of atopic eczema in at-risk children during the first two years of life. The preventive effect of the probiotic extended beyond infancy and was demonstrated also at 4 years of age [174]. *Lactobacillus rhamnosus* and *L. reuteri* administered for 6 weeks improved atopic dermatitis in children at $1 - 13$ years of age [175]. Similarly, *Lactobacillus GG* was effective in the treatment of children with atopic dermatitis probably caused by cow-milk allergy [176]. These reports suggest that probiotics may improve the composition of commensal microflora and prevent failure of the mucosal immune system to develop into a tolerogenic, non-inflammatory status. They represent a complementary therapeutic approach in atopic conditions and particularly the unique opportunity of primary prevention in risk subjects.

7.10 Critical Conditions and Abdominal Surgery

Theoretical and experimental prerequisites for the administration of probiotics in these conditions are at disposal. They include the decrease and elimination of pathogens and toxins, modulation of innate and acquired immunity, and the release of nutrient, antioxidant, growth, and other factors [177]. There is, however, a critical lack of valid clinical studies with indication outcomes. Jain *et al.* [178] applied a mixture of probiotics and oligofructose in critically ill subjects to improve the gut barrier function and to reduce the incidence of sepsis. The administration of the synbiotic favorably altered the microbial spectrum of the small intestine but did not affect the intestinal permeability and no clinical benefit was observed. The same authors described negative results with the preventive application of probiotics and oligofructose to patients before elective abdominal surgery [179]. On the other hand, Rayes *et al.* [180] reported significant decrease of postoperative bacterial infections and a shorter period of antibiotic administration after orthotopic liver transplantation in subjects with immediate postoperative enteral nutrition including a synbiotic (lactobacilli and oligofructose).

7.11 Immunization

Probiotics enhance the efficacy of peroral vaccines by different means. Oral vaccine of inactivated *S. typhi* administered together with *Lactobacillus GG* led to a more prominent increase of specific IgA as compared to placebo. *Lactobacillus lactis* and *L. reuteri* combined with the same vaccine increased the expression of CR3 receptor on neutrophils [181]. These findings suggest that individual probiotics influence the immune response in different ways following peroral vaccination, and that the immunomodulatory effect depends on the probiotic strain used.

7.12 Recombinant Probiotics

Live genetically modified probiotic strains offer new therapeutic possibilities. Recombinant *Lactobacillus plantarum* with a fragment of *Clostridium tetani* evokes a strong immune response on intranasal application [182]. The spores of *Bacillus subtilis* applied for the same purpose offer additional advantages: thermostability and easy genetic engineering [183]. Another important contribution may represent recombinant probiotics with good adhesion to intestinal epithelia and *in-situ* synthesis and secretion of therapeutic molecules. This approach has been already used with *Lactococcus lactis* (production and secretion of IL-10) [30, 31] and *E. coli* Nissle 1917 (production and secretion of an HIV-fusion-inhibitor peptide) [184]. The latter probiotic is considered to be a safe carrier of therapeutic molecules [185].

7.13 Dosage

The daily dose of a probiotic to convey a physiological or therapeutic effect is considered to be in the range of $10^8 - 10^{10}$ CFU. A more detailed assessment is hardly possible due to the fact that it is difficult to estimate the amount of viable microbes that may reach the target sites alive. This proportion depends largely on the pharmaceutical technology used. Protective coverings (e.g. pH-dependent acrylate resins, hydrophilic polysaccharides) are able to largely restrict the impairment of probiotics by digestive secretions and enzymes. This approach may indirectly increase the effect of prebiotics because their cleavage by probiotic enzymes takes place in the large intestine only. The complex structure of the majority of prebiotics (mixtures of polysaccharides with different molecular mass) makes it difficult to specify the individual components converted by microbial enzymes. The daily dose of the active prebiotic component (substrate) should amount to approximately $1 - 3$ g in children and $10 - 15$ g in adults.

7.14 Safety

The vast majority of probiotics are commensal nonpathogenic microorganisms, and significant undesired side effects occur very rarely as compared to other therapeutics used for the same indications. A detailed check-up on the safety profile of each microbial strain is a prerequisite for its use as a probiotic. In the clinical setting the risk of excessive translocation of the probiotic from the digestive tract into the systemic circulation should always be considered (e.g. in radiation therapy, bloody diarrhea, immunosuppression, and recent surgery of the oral cavity and digestive tract). In such situations the possibility of secondary infection should be evaluated (e.g. endocarditis, sepsis, or liver abscesses) [19]. More frequent *Lactobacillus GG* bacteremia has not been observed in connection with an increased use of this probiotic in Finland [186]. Prolonged administration of *S. boulardii* to immunosuppressed, polymorbid, and critically ill subjects deserves increased attention [187–189].

8 Prospect

The physiological character and safety of probiotic therapy allows to presume good compliance of the patients. A certain risk in self-treatment may exist by consuming different functional foods mostly with insufficient concentrations and variable quality of probiotics. Individual probiotics differ by their genetic equipment, properties, and effects. We need to know the optimal probiotic for the given patient and the stage of his disease (galenic form, method of application, dosage, and time of application). Further information is required on whether it is more suitable in a given case to choose a single probiotic strain or a mixture of several strains. At present the mixtures are considered acceptable if the individual strains are defined in sufficient detail [71].

Important prospective goals of probiotic therapy should include:

(1) Genomic analysis of probiotics, which would provide significant information about the proteomic potential and the functional capacity of the probiotic,
(2) Controlled postnatal colonization of the digestive tract with probiotics that may reduce the frequency of some diseases or improve their course,
(3) Use of probiotics as carriers of vaccines and therapeutic molecules with targeted application,
(4) Development of recombinant probiotics with in-situ production and targeted application of therapeutic molecules.

In view of the fact that the principle of the microbial interference therapy was formulated 100 years ago and marginalized for decades, it may be suggested that together with the development of theoretical and clinical disciplines the possibilities of probiotic therapy will be better utilized in the future.

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